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THE VITELLINE BODY IN SPIDER EGGS, ... *Nadine Nowlin.*

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THE VITELLINE BODY IN SPIDER EGGS.

BY NADINE NOWLIN.

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I. INTRODUCTION.

THE object of this work on the spider egg has been to gain some understanding of the peculiar element known as the vitelline body. Only those cell phenomena which in some way concern the origin, destiny and function of the body have been touched upon.

I want to express my thanks to Dr. C. E. McClung, who directed the work, and also to Mr. M. W. Blackman, for many helpful suggestions.

II. HISTORICAL STATEMENT.

There has been much confusion regarding the term "yolk nucleus." It has been applied to so many formations in the cytoplasm that its present meaning is very indefinite. Not only have we the one word applied to several different structures, but we are further embarrassed by having several different names for the same structure. Thus yolk-nucleus, corps vitellin, or vitelline body, and dotterkern are all applied to one structure by some writers, while sharp distinctions are made by others. The tendency now is to separate the terms, and call the peripheral globules, which collect in the early eggs just about the time of the yolk formation, yolk nuclei. Vitelline body is applied to the single large formation near the nucleus, arising also about the time of the collection of yolk.

The diffused yolk nuclei are of two kinds. Small bodies occurring very near the egg membrane, and staining densely, have been described by both Foot (4) and Calkins (2) in the earthworm. These occur in the egg of *Limulus*, as shown by Munson (6). The other diffused bodies lying in the cytoplasm, to which the term yolk nuclei has also been applied, are much larger than the peripheral formations, and lie scattered irregularly between the nucleus and the cell-wall. Calkins (2) in his work on *Lumbricus*, shows these bodies first collected in one mass around the nucleus, and later break up into separate spheres. They are homogeneous, and finally disintegrate, aiding in some way the yolk formation. Crampton (3) describes in *Molgula* a similar collection of material around the nucleus, and a subsequent breaking-up and scattering of the mass, though in this case the bodies are not so large or so definite as in *Lumbricus;* here the deutoplasm mingles more uniformly with the cytoplasm. We see, therefore, that while members of this class of yolk nuclei correspond to the peripheral ones in being many in number and in being scattered, still their origin is apparently different. The peripheral bodies certainly seem to arise in the cytoplasm ; at least, they are not a direct product of the nucleus. The others appear first close against the nucleus, and, according to Calkins, are formed from a portion of the chromatin reticulum cast out by the nucleus.

The position first occupied by the latter sort of diffused yolk nuclei would connect them with another class, the third and last of the deutoplasmic formations in the egg-cell. This is the single body which first arises as a crescent closely applied to the nucleus and later moves out from this membrane, becoming spherical in shape. Von Wittich was the first to investigate this body. In 1845 he observed it in the spider egg, and made a careful study of it in *Tegeneria domestica*. Balbiani (1) has perhaps done more work on this structure than any other one man. He made investigations upon both spider and myriapod eggs and found similar structures in them. In his paper "Centrosome et Dotterkern" he compares and identifies the two bodies.

He believed the yolk nucleus or "noyau vitellin" contained at its center the centrosome. In *Geophilus* (1) he found distinct radiations from that body which convinced him of the nature of the central sphere. In the spider he found no radiations, but the two formations agreed in other respects, and he concluded that in this class of animals, too, the yolk nucleus or vitelline body holds the centrosome. Munson (6) has described in much detail the egg of *Limulus*. He finds, also, the vitelline body there and compares it with that in *Geophilus*. Its position against the nuclear membrane, its striated outer wall, and its radiations are all similar. Munson believes the vitelline body of *Limulus* contains a centrosome.

The diffused yolk nuclei have been found by Jordan in amphibian eggs. Both the diffused and the single body, or vitelline body of Balbiani, have been found in mammals and birds (Mertens, 7), and in *Limulus* (Munson, 6). Whether these two apparently different classes of formations are essentially the same is a question to be solved by further investigation and comparison of different forms. The origin of the large vitelline body, as well as its fate, has been so variously given that nothing certain can be deduced. Largely for this reason its exact mission is unknown. Some have given it a very prominent place in the cell, believing it to be coordinate with the nucleus. Henneguy (5) compares it to the macronucleus of the Infusoria.

III. NOMENCLATURE.

The body found in the spider egg corresponds to the large single one in *Geophilus* and *Limulus*. For this reason I shall use the term *vitelline body* to designate it, and vitelline globules for the peripheral or scattered masses of yolk.

IV. MATERIAL AND MEANS OF COLLECTING.

Spiders are numerous and their eggs are still more so; for this reason I have been fortunate in not being limited in quantity of material. The early appearance of these animals in the spring and late disappearance in the fall is another advantage, as well as their habit of laying three or four times during the year. By or before the middle of March they may be found by turning up stones and old logs or by breaking off the loose bark of trees. The first warm days bring them out in great numbers. For my work, however, I could not go about picking them up indiscriminately, for some of the families do not have their eggs characterized by the vitelline body. The Agelenidæ, fast runners, but very handy to collect on account of their great numbers and showy homes, are among those without the structure. These spiders build the snowy, sheet-like web, so conspicuous on lawns and hedges, which possesses a tube, or funnel, through which the spider makes its retreat. Such spiders can be captured easily, however, by following down the tube, which is deceivingly short and frail at the bottom, and the captive makes little effort to escape when the tube is destroyed. Though these spiders have not the vitelline body, they are valuable for studying the form of the ovary; and in addition the eggs show the behavior of yolk free from the possible influence of a vitelline body. Their eggs are whitish in color, and possess a thinner membrane than those of the Lycosids, which is a favorable condition for fixation and embedding.

The Epeiridæ, that brilliantly colored family of orb-weavers, are, like the Agalenidæ, without the structure I sought. For this reason my study has been confined to two other families—Theridiidæ and Lycosidæ.

Therodion is the small, light brown or tan-colored spider

found so abundantly in cellars and other dark places. Members of this genus build irregular webs, and are convenient for collecting, since they can be found at nearly all times. They disappear very suddenly, however, when they realize an enemy is near; so I resorted to a means for their capture which proved successful in every case. By striking them slightly with the hard surface of the collecting bottle they drop from their webs upon the ground and feign death. There they lie with their legs drawn up about them, looking so nearly like a clod of dirt that one has difficulty in distinguishing them. They can easily be picked up from this position and placed in a bottle.

An important thing in collecting spiders is to have plenty of bottles or other receptacles for them. They are true cannibals, and should you place two together you will find but one in a short time.

My investigations have been made mainly on *Lycosæ*. These, on account of their great size and early appearance in spring, are especially desirable. The interest, too, attached to collecting them has perhaps caused me to use them. They are the very large, black, running spiders, inhabiting holes in the ground. They measure often one and one-half inches in body length and possess legs quite that long. Their mandibles are powerful and make no slight wound. They are, however, much more often on the defensive than the offensive, and I doubt exceedingly if they would attempt to do harm unless held or pained. They usually pull away from the enemy, even at the sacrifice of a leg.

Lycosa tegrina is the spider found about the University of Kansas. It makes its holes especially in the lower and flatter places, where vegetation is not too abundant and does not grow so tall as to hinder it running. I have found these holes most numerous where the land is low, moisture probably being a desired condition. The holes vary from an inch in diameter to one and a half or even two inches. They are from seven to nine inches deep, and do not, as some think, run straight down at right angles to the surface of the ground. I doubt if there is much uniformity in their methods or directions of digging, for those which I have examined have taken various turns. The main course is vertical, however. Often the opening runs obliquely for two inches and then turns downward. Classifica-

tion has at times been partially based on the manner of digging and the kind of hole dug, but it does not seem worthy of reliance. This species usually erects around the top of the hole a frail wall of web and grass.

Now as to the means for bringing the *Lycosidæ* from their holes something may be said. This is an easy matter if a little water be carried, and almost an impossibility without it. The water should be poured in slowly and allowed to run down the side of the hole. By this means the bottom begins to fill; as the water rises the spider climbs up to avoid getting wet, and soon appears at the top. If the water is suddenly poured in and strikes the inmate forcibly she becomes frightened and remains in the tube. Drowning her out is quite impossible, for it is well known how spiders can be submerged in water for days and still survive.

The males are easily distinguished from the females, not only because, as a rule, they have smaller bodies than the females of their species, and are usually more gaily colored, but because the male spider possesses attached to its head a pair of pedipalpi enlarged into bulbs at the ends. The female possesses pedipalpi, but no enlargement exists.

The greatest difficulty was experienced in obtaining the eggs in the maturation stages. This is because maturation takes place upon oviposition, and it is almost impossible to find them at just this time. For this reason, toward the close of March, I placed a large Lycosid in a glass jar half filled with well-packed earth. One day, so long after I had begun the experiment that I had decided it a failure, the animal began to dig near the center of the jar. When the hole was a half-inch deep she stopped removing the dirt and began to even the hole until it was very smooth with a gently curving bottom. Now she took a new position, with her feet on the edge of the opening and her body directly over it. From this position she lowered the abdomen until the spinnerets touched the center of the smooth surface beneath her and held them there for a short time. When she lifted the abdomen a small, shining patch of white was deposited on the ground and numerous silvery threads connected it with the spinnerets. This was the beginning of the cocoon.

From this time on she worked industriously. With a move-

ment as regular as clockwork the abdomen was raised and lowered—raised to unravel the threads, lowered and pressed firmly against the ground to fasten them. Never moving from the spot, but turning around and around over the hole, the little creature directed her spinnerets to every part of the bottom, until at last it was covered with a closely woven sheet. She was not content with the one layer, but spun another, and then touched it carefully here and there, to detect any neglected spots. When she found one, she spun diligently for some time about it, until it was completely remedied.

Then began the most interesting part of this queer performance. Placing the spinnerets at the edge of the white mat, she began once more a monotonous turning. So many times she turned and so empty the apparent result, I thought her insane from her previous work. After a long wait, I realized that a wall was growing, and although of such delicate and flimsy material, it was rising into space, unsupported by anything the eye could see. When completed, it was more remarkable still, for it leaned slightly over the hole, forming a sort of hollow sphere. A large opening was left at the top, however, and after a pause the spider ejected two drops of colorless fluid into the nest. These were soon followed by the eggs, which were a golden yellow, and so numerous and of such semifluid constituency that they were oviposited in a continuous stream. When the nest was almost filled, the little animal paused once more, then set about closing the opening. First she pressed the edges of the cocoon carefully over the eggs, but this not covering them, she began spinning once more. In a short time her treasures were sealed tightly within, and pressing the cocoon on all sides with the third pair of legs, she lifted it with the spinnerets and carried it to the edge of the jar.

V. TECHNICAL METHODS.

a. Fixation.

On account of physiological variations in the egg it was necessary to employ several fixatives. The very young ova are much more easily preserved than those further advanced; in fact, after the egg has entirely filled with yolk it is difficult to get a satisfactory fixation at all. The chief cause of the trouble lies in the egg-membrane. In the early follicular ova this is

very thin and delicate and is easily penetrated; but it increases in thickness as the cell grows, even adding in final stages new and separate layers, until days are sometimes required for the most penetrating fluid to reach the interior. Occasionally I succeeded in making an opening in the membrane by piercing it with a fine needle, but usually such attempts resulted in completely crushing the egg. One soon learns in working with such delicate material that the less it is handled the better. Even the force required to draw the eggs into a pipette will often break the membrane and allow the contents to flow out. To avoid these difficulties I left them in one vessel through the different processes, carefully drawing off the fluids from them and adding others. Even embedding was managed in this way; instead of lifting them from the clearing agent and placing them into melted paraffin, thus running the risk of crushing them at the very end of the process, I removed the fluid as completely as possible and then poured the paraffin upon them.

Of all the fixatives I used, Gilson's aceto-nitric sublimate mixture and Merkel's fluid proved most satisfactory. These came near working in all stages of the eggs' growth. Gilson's fluid was excellent for the oöcytes and even the mature egg, completely filled with yolk. The vitelline body shows at its best with this fixative, there being no shrinkage, and the material staining perfectly.

Merkel was used largely for the early stages, before the eggs had left the ovarian sac. The oögonia and young oöcytes are well preserved and stain readily with iron-hæmatoxylin. For this stage the Merkel fluid is better than Gilson, but those eggs in the follicle are not always successfully fixed. Passing this stage, however, Merkel works well once more; the mature egg is very well fixed by it, and also the segmenting egg.

Flemming has been used in preserving the ovary *in situ*, but not for a study of the finer structures.

Kleinberg's picro-sulphuric fixed the mature egg very well, but great care must be exercised in using it. If left in too long, the egg becomes greatly distorted and shrunken.

b. Stains.

Eggs fixed in Gilson and stained in Ehrlich's hæmatoxylin and acid fuchsin show the vitelline body very distinctly, but no radiations can be seen.

Lyons blue, with Gilson's fixation, makes a beautiful preparation. The eggs at all stages stain equally well with this. I have not found any stain which shows the vitelline body better. The yolk and cytoplasm take up the coloring, to be sure, but the vitelline body is much more deeply stained and stands out prominently. While the central sphere of the vitelline body is distinct, still the stain is not good for a study of fine structures.

For a study of mature eggs, Ehrlich's hæmatoxylin alone is very good. The yolk stains a uniform pale brown, while the nucleus and vitelline body are much deeper. The chromatic parts of the nucleus cannot be clearly distinguished, but the form of the nucleus at this stage can be seen. Younger eggs, even the oögonia, can be located clearly, but no careful study can be made.

Auerbach's stain, though the result varies with different conditions of the egg, differentiates the structures very well indeed. The young eggs color a delicate pink throughout. The yolk is yellow in those eggs where it has begun to accumulate; the nucleus and cytoplasm, pink. In other cases Auerbach stains all structures a purplish red, but even then the vitelline body is easily distinguished, because it is more densely stained.

Heidenhain's iron-hæmatoxylin is the best stain for detail work, as it brings out the most minute structures. Then the highest powers of the microscope can be used with this stain, while the diffuseness of the others makes such a thing almost impossible. For a study of karyokinesis I used it exclusively, and it did what the others failed to do; that is, it showed the pseudo-chromosomes, or small rods at the center of the vitelline body. It worked fairly well on mature eggs, but has the disadvantage of staining the yolk very densely, which in many cases cannot be bleached sufficiently for study. The thing most in its favor, however, is the uniformity with which it works, so that structures stained with it can readily be recognized at any stage of their development. While I used many other stains, I always verified my observations with this one.

VI. OVARY.

The ovaries of the spider are the most prominent organs of the abdomen. They lie completely embedded in a soft brown tissue, the so-called liver, more to the ventral than to the dorsal side of the body cavity. In the adult they extend from the anterior end of the abdomen to the posterior wall. As the eggs develop and increase in size, the ovary grows in thickness and width, until the entire abdomen becomes much enlarged. The liver grows proportionately thinner as this process continues. By a careful incision of the dorsal integument, and laying back the mass of liver, the numerous eggs may be seen. In *Lycosa* they are a rich yellow and very large, while in *Theridion* they are smaller and very white.

The ovaries thus filled with eggs have much the appearance of two bunches of grapes lying side by side. The entire mass is very soft, as the eggs have at this time no firm covering, but a most flexible and delicate membrane. Each ovary is connected at its anterior end with an oviduct, the ovaries themselves communicating at no point. Figure 1, plate XII, represents the posterior ends of two ovaries as they lie in the body cavity.

VII. OBSERVATIONS.

a. Development of the Ovary.

The reproductive organs of the spider differentiate at a very early period from the body cells, appearing first as two folds lying parallel to each other along the ventral aspect of the abdominal cavity. These assume a more definite shape as the animal grows, until there are soon two hollow cylindrical organs replacing the folds. The cells composing the ovaries at this early stage are apparently all alike, merely a mass of germinal epithelium surrounding a very small lumen. The outer wall consists of a layer of muscles, and between it and the epithelial lining lies a thin structureless membrane, the peritoneal coat, which cannot be distinguished until the follicles are formed.

So far the outer wall has a smooth surface, but very soon a change takes place on the interior of the tube which alters the appearance of the entire ovary. This change is as follows:

Certain groups of the cells lining the tube enlarge slightly and in time can be recognized as early oögonia. There are usually ten or twelve of the young germ-cells found clustered together, while around them the epithelium is unchanged. In one ovary such bunches may be very numerous. I shall not here describe the process in detail, but merely show how this change brings about the change in the form of the ovary. As these small germ-cells grow they push out through the muscular wall (Pl. XII, fig. 6), causing numerous diverticula on the outside of the tube. Projecting thus may be found oöcytes of nearly all ages, some very young, just pushing to the surface; others almost mature. By the time several generations of these cells have formed their follicles, the tube has become much distorted in shape. The walls in the old ovary are so convoluted that the lumen is difficult to distinguish. This is due to the great growth of the walls as well as to the distorting influence just mentioned.

For a study of the morphology of the ovary a young specimen is much better than an old one. The ovary has more rigidity at this time and is a straight tube, of which it is very easy to obtain a longitudinal section. A study of the organs *in situ* is perhaps more satisfactory than any other way, giving us best their relation to each other in the body. Fleming is very good as a fixative for this purpose, since it will penetrate even the tough outer covering of the body. The surest way is, however, to remove this covering before placing the fluid upon it. The fixative will then readily penetrate the tissue of the liver and will fix the ovary perfectly.

There is another advantage, too, in working with the young ovary. The ovarian cells and those in the follicles have not differed to any extent physiologically, and all come out equally well preserved. In the mature ovary, those eggs which have becme filled with yolk take the stains so differently from the younger eggs, especially the cells of the ovarian wall, that in preparation one set must be completely sacrificed for the other. It is almost impossible to make longisections of the distorted adult ovary. Cross and oblique sections quite as often occur, producing some very confusing images to one unacquainted with the structure of these organs.

b. Development of the Oöcytes.

The spots along the ovarian lining at which a conversion from epithelial into germ cells is taking place can easily be distinguished. At such points there is a great thickening of the lining, or, we might say, a great multiplication of epithelial cells has taken place, until the new cells project far out into the lumen of the tube (Pl. XII, fig. 1, *c*, *d*, *e*), sometimes even filling the opening and crowding against the opposite wall. These cells, at their earliest stage, do not differ in appearance from the epithelial cells around them, unless it be in the nucleus, which seems to be slightly larger and richer in chromatin. The form of both, however, is much the same. Soon the nucleus increases in size until it almost fills the cell, the cytoplasm growing some, though not in proportion to the germinal vesicle. Out of the indefinite masses of chromatin a slender spireme is formed, which seems to consist of many long threads, crossing and winding among one another. At what appears to be the extremity of each thread is an enlargement which stains very densely. A close examination of these threads shows them to be made up of small segments, placed end to end. (Figs. 4, 5, 6, pl. XII.)

The stages between the spireme and the early oöcyte were clearly seen in ovaries fixed in Merkel's fluid, though much material collected was lacking entirely in cells at this stage. Lying embedded in the epithelial lining could be found division figures at all stages. (Pl. XII, fig. 2.) The chromosomes were distinct, but much crowded, so that, while I counted nineteen, I am by no means sure this is the actual number. The cells grow rapidly from this period, and their development is easily traced. They are always distinguishable by their clear appearance; large nuclei, with scattered patches of dimly stained chromatin, and cytoplasm, thin and lacking in reticular structure. (Fig. 7, pl. XII.)

Though in the early oögonial stages the developing germ-cells keep even pace, in their later development one gains decided advantage and grows much more rapidly than the others. We seldom find a group of oöcytes equally developed, but much more often we see one far advanced lying among many oögonia, probably in the spireme stage. It seems that natural selection has come into play, choosing one from the many

of equal age, and if not developing this one at the cost of the others, at least giving it much advantage in point of time. (Fig. 7, pl. XII.)

The location of the oöcyte changes as it grows, the cell moving more and more to the periphery of the tube, pushing through the epithelial lining, and finally through the muscular coat upon the exterior. (Fig. 6, pl. XII.) Its connection with the tube is not severed, however, for it has not broken the peritoneal coat. It has merely pushed this before it through the opening it has made in the muscular wall. Besides this means of preserving its relation to the ovary, it has a more efficient one still: a small mass of epithelial cells seems to follow in the track of the egg and to attach one point of its periphery. These cells are pulled partly out through the opening, causing them to appear as a neck holding the oöcyte in place. Their real mission is doubtless to convey nourishment from the interior of the ovary to the growing oöcyte, which needs such quantities at this period in its development.

The appearance of the nucleus during this progress does not materially change. While the oöcyte is in the tube the nucleus contains little chromatin, and this almost immediately begins to collect together. (Fig. 7, pl. XII). The cytoplasm is scanty and is scattered irregularly through the cell.

From this time the egg is most interesting in its development. The wall around it becomes thicker and firmer, yet does not lose its elasticity; for rapid growth takes place, enlarging and expanding constantly the outer membrane. The cytoplasm of the egg is no longer clear, but there seems to be an inpour of material clouding the cell and forming in certain parts of it a dense zone. There appears about this time, too, the peculiar element known as the vitelline body, the growth and development of which is unique. These things will be dealt with in detail later, so I merely mention them here.

As the cell grows the nucleus increases in size, becoming richer in chromatin. It possesses always one very large nucleolus, and very often three or four smaller bodies. The nucleolus is always vacuolated and the small bodies lying near have very much the appearance of being given off from it, as Balbiani described in his work on *Tegenaria*. Occasionally there may be seen inside the nucleolus very dense opaque bodies or

disks which do not stain, but lie bright and shining, refracting the light to a much greater degree than the surrounding structures. (Fig. 20, pl. XIV.)

c. The Vitelline body.

Up to the time when the oöcyte has left the ovarian sac, there is no trace of a vitelline-body; but the earliest follicular eggs contain what I take for the beginning of its formation. (Fig. 1, *g*, pl. XII.) Close against the nuclear membrane, and at one side, granules begin to collect, as if the cytoplasm were condensing at this point. The mass is at first scanty and extends only partially around the nucleus, forming a thin, crescent-shaped body; the granules are now very coarse and far apart. But as the crescent widens or increases in size the granules aggregate more closely, becoming quite dense at the point near the nuclear membrane where they first collected. Before this zone of dark granules has extended entirely around the nucleus, careful focusing will show a small sphere at its densest point. (Fig. 10, *a*, pl. XIII.) This appears to be a simple capsule; at least there are no concentric layers as seen later. I am not certain of the internal structure of the body at this early age, but it is probably even at this time more complex than it appears, as I was able later, when the surrounding granules are less dense, to make out definite granules within. The usual appearance, however, and especially in its early stages, is a clear sphere, with a thin but well-defined surrounding membrane.

The crescent increases in size until its horns often meet on the opposite side of the nucleus (Fig. 11, pl. XIII.) This indicates an increasing amount of the granular deposit, which is doubtless a food material. As the mass thickens it forms into layers around the central sphere, the layers varying in number, width, and density. The number indicates to some extent the age of the body, the larger and consequently the older eggs having a greater number of rings than the smaller. (Fig. 12, pl. XIII.)

Fixations and stains have much to do with the appearance of the vitelline body; some obscure the concentric layers completely and cause the structure to look like a homogeneous mass. (Pl. XII, fig. 9.) Often, however, in the best-stained material the body varies, and I am inclined to believe that variations in appearance are not always due to stains and fixa-

tives, but frequently to the body itself. Very often when the concentrically striated wall is most distinct no central sphere can be seen, and there is a question in my mind whether it is always present. Merkel's fluid is best for showing the central sphere. Stained with iron-hæmatoxylin, it appears as a clear, transparent body with a definite outline, embedded in a homogeneous, densely staining mass, which is surrounded by a still denser ring. In some cases the sphere contains granules which stain much like chromatin. Again, in material fixed in Gilson may be found definite rod-shaped bodies in the central sphere of the vitelline body. (Pl. XIII, fig. 18.) Sometimes, when fixation has proceeded too far, the granules surrounding the central disk will draw together into more compact layers, thus separating slightly from the sphere and also from one another. There can then be seen a network of fibers throughout the vitelline substance, indicating that the latter simply lies embedded in the cytoplasmic reticulum. (Pl. XIII, fig. 12.) There is at times a suspicion of radially arranged fibers issuing from the central vesicle, but it is by no means distinct, and is probably no more than the network just described. (Fig. 26, pl. XIV.)

Later appearance of the vitelline body.—The vitelline body lies, first, closely applied to the nucleus, even at times indenting its wall. (Fig. 9, pl. XII.) As the dense zone around it clears up, the body moves out from the nucleus, and for a time gets farther and farther away. This is during the period of greatest cytoplasmic growth, and the separation of the two bodies seems to be influenced by it. Its distance from the germinal vesicle varies in different eggs, but usually, during the yolk formation, it lies near the periphery of the cell, separated from the nucleus by the entire radius of the cell. (Fig. 28, pl. XV.) The appearance of the body at this time is often peculiar. The central sphere is not distinguishable, and in many respects the body appears to be disintegrating. Figure 28, plate XV, shows the vitelline body lying near the periphery of the cell; the outline is definite on all sides but one, and here it fades into the cytoplasm, as if becoming a part of it. The body is no longer composed of concentric layers, but the granules which formerly constituted these seem to have formed into definite small, spherical bodies, much resembling the small globules of yolk, or

yolk nuclei of Foot and others, which are at this time forming at the periphery of the egg. These small masses, which stain a faded brown in iron-hæmatoxylin, are bursting from the broken wall of the vitelline body and are becoming scattered through the cytoplasm in that neighborhood. The material which showed this is some of the best preserved that I have, so it would not seem to be due to faulty action of the fixing agent. No central vesicle can be seen; the entire mass seems simply to be giving way and mingling with the cytoplasm.

Figure 29, plate XV, shows another appearance of the body in a late oöcyte. Here the concentric layers are disintegrating, only traces of them remaining. The contents have become coarsely granular and show a vacuolated or perforated condition. Figure 30, plate XV, exhibits the vitelline body of another egg, almost ready to leave the follicle. It is surrounded by yolk-spheres and cytoplasm. It shows, also, many vacuoles, and has much the appearance of a disintegrating body.

Had I stopped with these observations, I should certainly believe the body completes its mission as a food-producing organ thus early in the egg's history and then disappears. I am not sure but what it does this in some cases, as I can account in no other way for the appearance just described. It may be that the constitution of the egg differs enough to affect the persistence of such a body, causing it to disintegrate sooner in some than in others. However this may be, I found the body in other material, and in eggs much farther advanced than those just mentioned, still quite definite. Figure 31, plate XV, shows an egg entirely filled with yolk. The nucleus lies near the center of the cell, and very close beside it we see the vitelline body. The concentric layers are still present, though not as distinct as at first; the central vesicle is visible. The cytoplasm of the egg is reduced to the space immediately surrounding the vitelline body, and connects the latter with the nucleus. Iron-hæmatoxylin stains the yolk masses too densely to allow any structure to be distinguished. By much decolorizing the nucleus sometimes will give up the stain enough to become visible. The best stain for eggs at this stage, however, is Ehrlich's mixture.

We see, then, that the vitelline body, which has been widely separated from the nucleus, is once more drawing toward it, and at the time just previous to maturation. This seems to

favor the theory that the central clear vesicle is the centrosome, and approaches the nucleus preparatory to the maturation divisions. But let us follow the body further. We are able to trace it up to the time the nucleus breaks up for the maturation divisions, and, for a period, nothing is plain; the great massing of yolk has obscured all other structures. It would be most interesting to be able to follow the vitelline body through this stage, though I am convinced it takes no part in division. This being a centrolecithal egg, the nucleus and scanty mass of cytoplasm surrounding it have moved to the periphery of the cell, and there divide, forming a layer of new cells just inside the old cell-wall. The yolk remains undisturbed at the center. The new cells formed by the first few divisions of the mother nucleus contain no trace of anything similar to the vitelline body; that is, the embryonic cells do not possess the structure. But if we look carefully through the central mass of yolk we shall find the vitelline body almost as perfect as before maturation. This indicates that it played no part in the maturation processes, and especially not that of a centrosome.

Its disappearance varies; usually it is gone before quite all the yolk globules are absorbed by the growing embryo.

d. The Vitelline Zone.

One of the most noticeable feature of many of these follicular eggs is the granular area, called by Balbiani the vitelline zone. In my material I find zones of two kinds. Always there appears the cloud around the nucleus previous to the vitelline body formation. This grows and spreads out considerably into the egg cytoplasm, usually remaining densest around the nuclear membrane, and gradually blends with the reticulum. The granules composing this zone, as a rule, stain more deeply than the cytoplasm, though I have sometimes found the very opposite true. The other zone is perhaps even more marked than this one. It is not found in all eggs, but chiefly in those of the young ovaries. The egg at this time appears to be composed of two parts, an inner sphere, filling about three-fourths of the egg, and an outer lamella. The former is of closely packed granules, with no reticular structure, and stains densely; the latter is not greatly different, but is much clearer and thinner, with a suspicion of reticulum. There is no gradual blending

of these two zones, as in the crescent-shaped one first described. A definite wall separates the inner from the outer layer.

There is one feature of this zone that gives us, perhaps, the safest clue to its origin and composition. Plate XIV, figure 22, shows a section through the long axis of the egg and egg-stalk, and we see the inner zone extending into the epithelial cells of the stem, or, more according to my belief, rising from these epithelial cells. Now, this zone must be the food supply that is being constantly brought in by these nurse-cells in the egg-stalk, and deposited in the cytoplasmic network so compactly that the latter structure is made invisible. In the neighborhood of the vitelline body the mass stains more densely, showing a gathering of the metaplasm especially at this point.

The vitelline zone of Balbiani, or the dense cloud which collects about the vitelline body, and is especially prominent in the later stages of the follicular egg, has its origin also from the food material brought into the oöcyte by the nurse-cells, and is probably but a condensed form of the larger zone so conspicuous in the early eggs.

e. The Egg-stalk.

The above leads to a discussion of the function of those cells in the stalk of the follicular egg. We saw how the young oöcyte lay first among a group of growing oöcytes within the wall of the ovary. (Fig. 1, *e*, pl. XII.) For a time all remained equal in size; then, from some advantage which one or two gained over the others, they developed much more rapidly and completely outgrew them. As these increase in size they crowd through those around them, press up through the epithelial layer, and even separate the muscular covering, to pass through. The egg upon leaving the epithelial layer of cells comes in contact with the peritoneal membrane, which it cannot pierce; but the membrane, being flexible, is pressed before it through the muscular wall and there holds the egg in place. The epithelial cells, having been displaced, now move back and fill all space made by the departing oöcyte, shoving close against the lower wall of that cell. The arrangement of the cells would naturally be altered and they would no longer be so closely packed together, thus explaining the definite outline of the stem or yolk-stalk. (Fig. 1, pl. XII.)

These epithelial cells no doubt serve as nurse-cells to the follicular egg. The wall is not well defined around those oöcytes which have just pushed to the surface, and the nurse-cells press into their cytoplasm for some distance. (*G*, fig. 1.) We find this in still older eggs. Figure 16, plate XIII, shows the epithelial cells of the stalk lying in the cytoplasm of the oöcyte, entirely surrounded by it in some cases. Whether this is carried to a still greater degree and the cells absorbed by the cytoplasm, as has been suggested by some authors in other material, I cannot say, but figure 27, plate XIV, shows a gradual fading of these cells, which would indicate that some, at least, are being dissolved. As the egg grows toward maturity, and its food supply is about complete, a definite membrane forms between the neck and the contents of the egg. Figure 24, plate XIV, shows the complete formation of such a membrane, and also the reduced size of the stalk. The epithelial cells have, I believe, completed their mission, and the space which they occupy in the ovarian wall is assuming its former appearance. Figure 32 shows the neck about to disappear.

f. Eggs without the Vitelline Body.

Those eggs which do not contain the vitelline body present some differences. The cytoplasm of the young eggs contains much scattered food material. (Fig. 35, pl. XV.) Also the yolk formation begins much earlier than in eggs having the vitelline body.

VIII. SUMMARY AND INTERPRETATION.

The vitelline body makes its appearance first in the young oöcyte. After the last oögonial division the cell membrane is reconstructed, and for a time the cell grows rapidly. When it has attained a certain size it moves to the periphery of the tube preparatory to forming the follicle.

The oöcyte is occupied for a time in making its way through the ovarian wall. When it is at last upon the exterior, attached by a number of nurse-cells to the tube, a small crescent of darkly staining granules can be seen around the nucleus. At a slightly later stage a denser spot in the form of a tiny sphere can be observed in the midst of the granules.

Let us try to explain the phenomena thus far observed: the

dark zone, the denser spot at the center, and, finally, their appearance just at this time. The dark crescent is beyond doubt a collection of food material. Its origin may be, in part, from the nucleus, but mainly, we believe, from the substances brought in by the nurse-cells. Either the chemical composition of this material is such that it is not broken down directly and used, or more food-substance is furnished than the cell can utilize. As new supplies are constantly going in, the pressure becomes greater, and the granular mass grows more dense. Finally, close against the nuclear membrane at the widest portion of the crescent, and, consequently, at the point of greatest pressure, a spherical mass appears, the vitelline body. I believe the time of appearance of this structure depends upon the supply of food material to the cell.

The central clear vesicle is perhaps most perplexing. Balbiani (1) and Munson (6) believe it either to be, or to contain, the centrosome. The former regards it as a functionless centrosome of the female cell, but the latter considers it the centrosome of the dividing oögonia. True, its appearance is different from the surrounding layers of granules, yet it resembles so little a centrosome that appearance will not help us much in discovering its function. The best evidence that it is not the centrosome is its frequent absence, while all other parts of the structure are perfect. In such cases the central space is merely a loose collection of granules, like those of the concentric layers. This leads me to believe that this central clear vesicle is the result of great pressure of the metaplasm, which might bring about an extraction of certain constituents of the cytoplasm, and these, pushed thus to the center, form the central sphere of the vitelline body.

The body next moves from the nuclear membrane, which movement is probably due to increase of metaplasm between the two, or else to a growth of the cytoplasm.

Yolk now appears in the egg, usually about the periphery, in the form of "yolk-nuclei," described by Foot. In some cases the vitelline body seems to be disintegrating at this stage, breaking up directly into small globules of yolk. This early disappearance is another reason for believing it merely a food body. True, the surrounding metaplasm could disappear at this time and leave the central vesicle to perform its centro-

some function unencumbered; but it is a complete giving way, central vesicle and all.

The yolk, pressing more and more to the center, drives the nucleus and vitelline body inward, until we find them again close together, connected by the scanty remnant of cytoplasm which persists. Of course, this could take place only in those eggs where the vitelline body does not disintegrate, and, as a rule, the structure persists very long after this stage.

Just before maturation the nucleus moves to the periphery of the cell, leaving the vitelline body at the center. Here the nucleus divides, unaided in any way by the structure, which Munson believes to be the "centrosome of the growing oöcyte," and, lying undisturbed at the center of the cell, can be seen the vitelline body, even after numerous layers of cells have been formed. Balbiani found the body distinct in embryos two weeks old. The body fades when the yolk is about to be exhausted, or, we might say, is finally absorbed along with the yolk by the cells of the developing embryo.

This convinces me that the vitelline body contains no centrosome, but is merely a condensation of certain food-substances, which the protoplasm of the cells of certain spiders cannot immediately break down and use. Its absence in some families is due to those cells possessing engemes sufficient to dissolve these substances more rapidly.

More than this, structures identical with the central vesicle of the vitelline body have been found in eggs which at the same time possess perfectly distinct vitelline bodies. (Fig. 21, pl. XIV, and fig. 27.)

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X. PLATES AND EXPLANATION.

Drawings were made with a *camera lucida.* A Bausch & Lomb one-sixth-inch objective and one-inch eyepiece were used, except in figures 2, 6, 7, 8, 17, 29, where a one-twelfth-inch objective was employed. Occasionally a still greater magnification has been obtained with a one-half-inch eyepiece, viz., figures 3, 4, 5, 25, 32.

Zoological Laboratory,
University of Kansas,
June 1903.

TYPE VI.

(7)	${}^6G_1(ll'mm'nF)$, or ${}^6G_1(APps_mF)$.	Two generators of each system, a pair of reciprocal polar lines, and all points on one of those lines. (VI. $E_{c=1}$)
(8)	${}^6G_2(ll'mF)$.	Two generators of one system, and one of other system. (V. $C_{c=1}$)
(9)	${}^6G_3(lmF)$.	One generator of each system. (IV. $D_{c=1}$)
(10)	${}^6G_2(APpsF)$.	A point on the surface, tangent plane at that point; a point without surface and its polar plane. (V. D)
(11)	${}^6G_3(PsF)$.	Point and polar plane. (IV. E)

TYPE III.

A.—Groups of first class.

(12)	${}^3G_2(ll'mF)$.	Two generators of one system, one generator of other system. (V. F)
(13)	${}^3G_3(lmF)$.	One generator of each system. (IV. B)
(14)	${}^3G_4(lF)$.	One generator of one system. (III. A)

B.—Groups of second class.

(15)	${}^3G_1(ll'mF)_a$.	Same as ${}^3G_2(ll'mF)$ and constant a. (VI. C)
(16)	${}^3G_2(lmF)_a$.	Same as ${}^3G_3(lmF)$ and constant a. (V. B)

TYPE I.

A.—Groups of first class.

(17)	${}^1G_2(ll'mm'F)$.	Two generators of each system. (V. G)
(18)	${}^1G_3(ll'mF)$.	Two generators of one system, and one of the other system. (IV. C)
(19)	${}^1G_4(ll'F)$.	Two generators of one system. (III. B)
(20)	${}^1G_4(lmF)$.	One generator of each system. (III. C)
(21)	${}^1G_5(lF)$.	One generator of one system. (II)
(22)	${}^1G_6(F)$	None. (I)

B.—Groups of second class.

(23)	${}^1G_1(ll'mm'F)_r$.	Same as ${}^1G_2(ll'mm'F)$ and constant r. (VI. E)
(24)	${}^1G_2(ll'mF)_r$.	Same as ${}^1G_3(ll'mF)$ and constant r. (V. C)
(25)	${}^1G_3(lmF)_r$.	Same as ${}^1G_4(lmF)$ and constant r. (IV. D)
(26)		Identical transformation. (VII)

PLATE XII.

FIG. 1.—A longitudinal section through the ovaries of a young *Lycosa*, showing the posterior ends of these organs as they lie in the body cavity. The ovary is a hollow cylinder lined with epithelial cells, *a*, and covered with a layer of muscular cells, *b*. At several points along the tube can be seen numerous cells projecting from the wall and almost, or quite, filling the lumen of the tube. These are the germ-cells which have differentiated from the epithelial lining; *c*, the very early oögonia, as yet scarcely differentiated; *d*, oögonia in the spireme stage; *e*, oögonia in the spireme stage and one oöcyte; *f*, follicular oöcytes, or those which have pushed through the wall upon the exterior of the tube.

FIG. 2.—Oögonia dividing to form the oöcytes. Though small and embedded in the cells of the ovarian wall, these division figures are easily distinguished by their clearness as compared with the granular appearance of the surrounding cells.

FIGS. 3, 4, 5.—Greatly enlarged oögonia, showing the formation of the spireme.

FIG. 6.—A small portion of the ovarian wall, showing three oöcytes, *a*, pushing their way through the epithelial lining, *b*; *c*, a well-developed oöcyte upon exterior of ovary.

FIG. 7.—Portion of ovarian wall, showing muscle cells, *a*, and epithelial cells, *b*. Numerous oögonia, *c*, in the spireme stage surround the oöcyte, *d*. It is probable that this cell is no older than the sister cells among which it lies, but on account of some advantage in food supply has developed in advance of them.

FIG. 8.—Two oöcytes, *a* and *b*, lying against the ovarian wall, *c*, showing the relatively large size of the nucleus at this stage and its scattered masses of chromatin.

FIG. 9.—A follicular oöcyte, showing the large germinal vesicle, *a*, and the vitelline body, *b*, indenting its membrane. The fine granular area, *c*, is the vitelline zone in which lies much food material. The outer reticular area, *d*, is the cytoplasm. Comparing this figure with *f*, figure 1, we see the growth the egg makes as it nears maturity.

PLATE XII.

PLATE XIII.

FIG. 10.—Young follicular oöcyte in which the vitelline body, *a*, is developing. Here is seen in this stage scarcely more than the central vesicle, the whole clouded by a zone of darkly staining granules. The body is distinctly a part of this zone. *b* is the germinal vesicle and *c* the stem of epithelial cells which attaches the egg to the ovary.

FIG. 11.—An oöcyte somewhat older than at figure 10, in which the vitelline body is assuming a concentrically striated wall. The clear central part of the body is the central vesicle, though it possesses no well-defined wall, as in figure 10. Near the periphery is a vacuole, *a*.

FIG. 12.—The vitelline-body has moved out from the nuclear wall. It not only lies in the vitelline-zone, but is closely surrounded by a dense layer of granules. This is probably food material which it is extracting from the zone and is storing up. Between the periphery of the vitelline body and the central mass is a coarse network, which I take to be no more than the cytoplasmic reticulum made prominent by the deutoplasmic granules which adhere to it.

FIG. 13.—The vitelline body is now separated from the germinal vesicle by almost the diameter of the cell. It here possesses a clearly defined outer edge, which separates it sharply from the surrounding structures.

FIG. 14.—Instead of a body formed of concentric layers, there is a collection of large globules which stain very black in iron-hæmatoxylin. Those at the center are in the shape of rods, and resemble chromosomes.

FIG. 15.—A follicular egg, showing the egg-stalk, *a*, attaching the large oöcyte to the ovarian wall. The vitelline body possesses a wall spirally wound about the central vesicle.

FIG. 16.—The germinal vesicles of eggs at this stage of development possess very large nucleoli, which are usually vacuolated. The smaller bodies stain darker, and have much the appearance of being cast from the nucleolus.

FIG. 17.—A highly magnified vitelline body, showing its well-defined outer edge and the network of fibers within.

FIG. 18.—A follicular oöcyte, showing the peritoneal coat, *a*. Here are found again some new characteristics in the vitelline body. The central vesicle is comparatively large and distinct, and instead of being clear is filled with darkly staining rods, or pseudo-chromosomes.

FIG. 19.—There is shown here a condition which is very rare. The cytoplasm surrounding the vitelline body is faintly radially striated.

PLATE XIII.

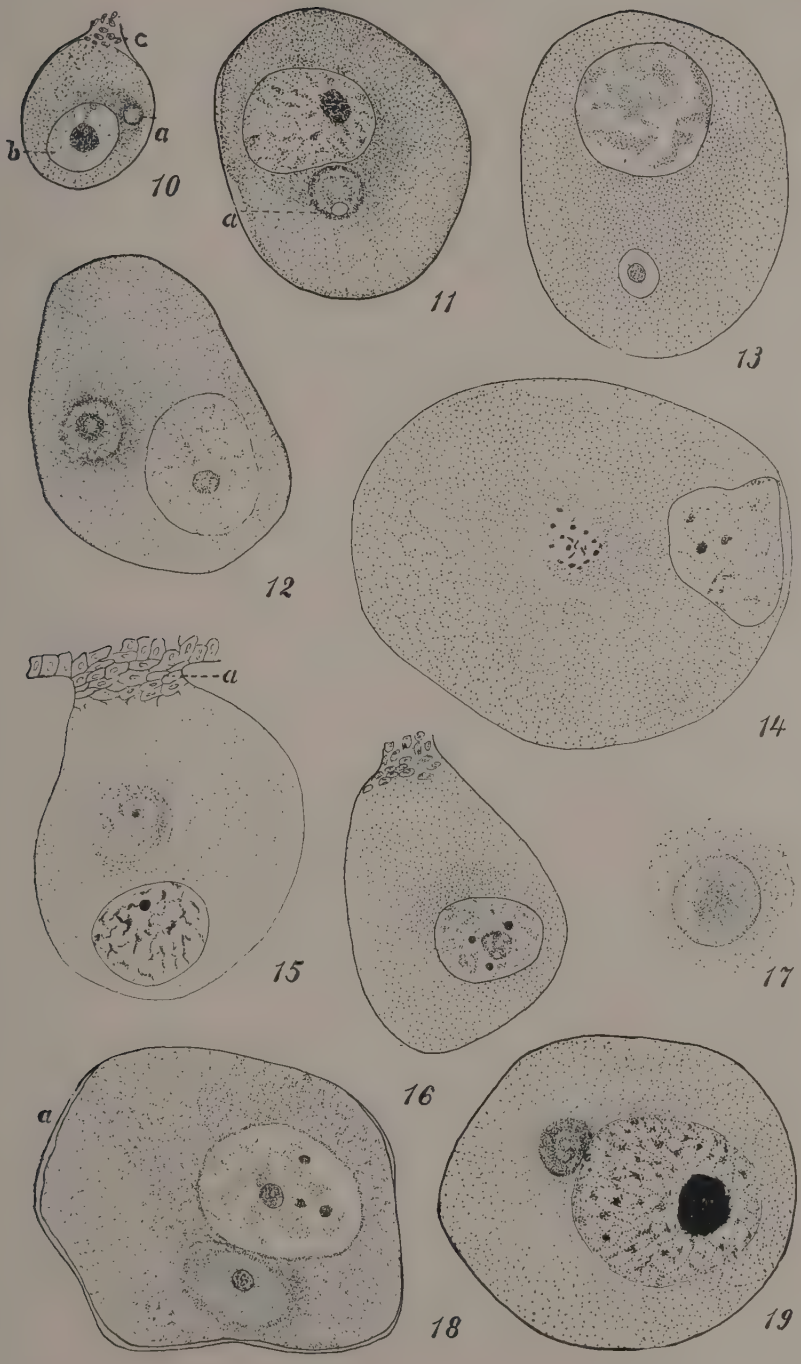


PLATE XIV.

FIG. 20.—The vitelline body shows a distinct central vesicle, and a striated capsule, the outermost layer touching the nuclear wall. The cytoplasm seems to be drawn toward the body from all directions. The nucleolus is very characteristic of the nucleus just now. The clear vesicle within is filled with very dense bodies, which do not take the stain, but shine with a metallic luster.

FIG. 21.—The cytoplasm throughout this egg is clear and uniformly reticular. At *a* is the vitelline body. At *b* and *c* are structures identical with the central vesicle of figure 18, plate XIII; *b* is especially clear, with a well-defined wall and equally well-defined pseudo-chromosomes within. This multiplication of the structure which we have called the central vesicle seems to show that it is not confined to the vitelline body, and, moreover, seems ample proof that it is not the centrosome of the spider egg.

FIG. 22.—The vitelline zone, *a*, is conspicuous in the early follicular eggs. It extends from the nurse-cells in the stalk almost to the periphery of the egg.

FIG. 23.—A peculiar appearance of the oöcyte, in which the yolk globules, *a*, have begun to form about midway between the periphery and the center.

FIG. 24.—An oöcyte almost ready to leave the follicle. The stalk, *a*, is very small and the nurse-cells few and reduced in size. A membrane, *b*, seems to be forming between the nurse-cells and the egg contents.

FIG. 25.—Two oöcytes just previous to forming the follicle. There is at this time no trace of a vitelline body.

FIG. 26.—An oöcyte in which the concentrically striated capsule of the vitelline body is very distinct. At the center may be seen a sphere much smaller than any yet found. There seems to be little uniformity in size of this formation, which fact throws doubt upon its being a definite structure.

FIG. 27.—Here again, as in figure 21, are two structures, besides the perfectly developed vitelline body, simulating the central vesicle.

PLATE XIV.

PLATE XV.

FIG. 28.—Yolk is beginning to form at the periphery of the egg. *a*, yolk globules; *b*, the yolk-nuclei of Foot; at *c* we meet with a peculiar condition of the vitelline body. The structure no longer retains its spherical form, but shows one side broken away and the contents scattered. The body has every appearance of disintegrating.

FIG. 29.—The vitelline body much vacuolated. There is merely a remnant of the concentric layers left, and the entire structure seems to be dissolving and mingling with the cytoplasm.

FIG. 30.—Another vitelline body which shows no central vesicle. The structure has a spongy appearance and no concentrically arranged border. *a* and *b*, yolk globules.

FIG. 31.—Oöcyte completely filled with yolk. The nucleus and vitelline body are once more close together, connected by the scanty cytoplasm which remains in the cell. *a*, yolk globules; *b*, nucleus; *c*, vitelline body.

FIG. 32.—Egg about ready to leave the follicle. The neck is rapidly diminishing and the egg membrance is forming at *c*. The entire egg is at this time filled with yolk, *a*, and the nucleus is close against the cell-wall, this being the position of nuclei just before maturation in centrolecithal eggs.

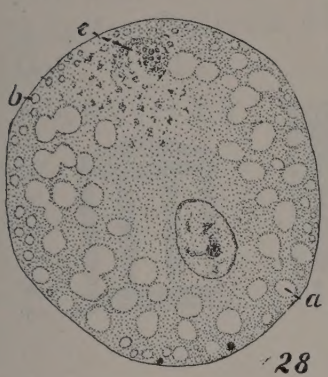
FIG. 33.—Portion of another egg preparing for maturation. There is no trace of the vitelline body applied to the nucleus, as was found a little earlier in figure 31. The assumption is that it has been left behind, though at this stage so much yolk obscures it.

FIG. 34.—The assumption concerning the vitelline body of figure 33 is proven true by this figure. Here is a segmenting egg. The nucleus at the periphery has divided, forming the cells, *a*. At the center, among the yolk globules, we find the vitelline body, *b*, unchanged.

FIG. 35.—A follicular egg of *Epeira*, which represents those eggs without the vitelline body formation. Numerous patches of food material cloud the cytoplasm.

FIG. 36.—Highly magnified vitelline body, showing the central vesicle, *a*, and the rod-like structures within.

PLATE XV.



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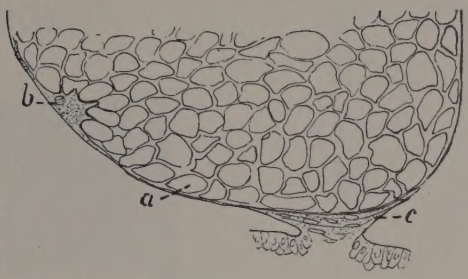

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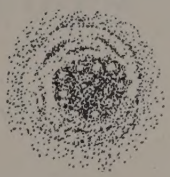

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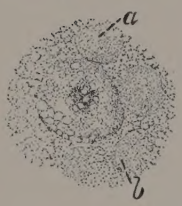

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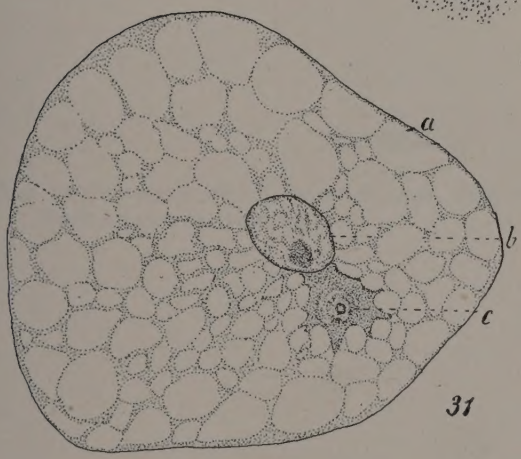

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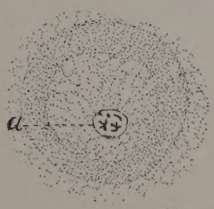

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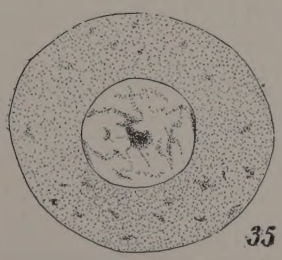

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